# iGEM UNOTT 2017 Freeze Drying Protocol

- Bacteria grown in as a normal overnight culture.
- Growth curve undertaken (standard one), samples are taken at just before stationary phase at around 6 - 8 hours.
- Samples of taken and transferred into a 2ml eppendorf tube and spin down, discard the supernatant. Resuspended in sterile water, then spin down again.
- Final resuspension done in 300ul sterile 10% sucrose solution. This is the cyroprotectant. It builds up inside the cell to stop it collapsing when desiccated. It keeps cell structure.
- These were left for a bit (5 mins) to allow cyropretectant to build in cell.
- Tubes and sample were snapfrozen in liquid nitrogen (initial protocol with test devices were snapfrozen in dry ice) and then placed in dry ice.
- The tubes were then uncovered and wrapped in parafilm and pierced with a toothpick or the lids were pierced with a scalpel (sp4 and wp1 were done this way) to allow the moisture to escape later.
- These were then placed in chilled vacuum pump machine. They were left overnight to allow any moisture to be removed, leaving a “dust” of freeze dried bacteria.
- These were then covered again. They are covered so that oxygen doesn’t meet it. When not stored at -80°C oxygen builds up and causes oxidative damage to cells which reducing viable cells.
- Cells revived with 30°C SOC for faster revival.
- Next you can measure to find out how many cells survived!